TRANSDUCTION OF H-ANTIGEN BETWEEN H_1-DUPLICATE STRAINS.

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The H-antigens a and c in SW1053 (a:c), and b and 1,2 in SW1043 (b:1,2) have been assumed to be controlled by different H_1 loci in each; that is, the H-antigen factores of SW1053 are composed of $H_1^a H_1^c$, and that of SW1043 are composed of $H_1^b H_1^{1,2}$. Each of these four loci have been proved to be transduced to the H_1-locus of H_1H_2-diphasic strains, linked with Fla_{666} in some frequencies, but not be transduced simultaneously.

The present experiment is to test whether simultaneous transduction occur or not when transduction of H-antigen is made between above two strains; that is, both donor and recipients are H_1-duplicated diphasic strains.

EXPERIMENTS.

The transduction experiments were done in both directions; using a-phase SW1053 and b-phase SW1043. The doner lysate and the overnight penassay broth cultures of the recipient were mixed in equall volume, and ca. 0.05ml of them were inoculated on the top of each MGA-stabs which contains H-antisera to the recipient cultures. As controls, lysates and recipient cultures were dropped separately, and also the recipient cultures were dropped on the MGA-stab without antiserum. They were incubated at 37C and watched the growth of swarms. When swarms grew, they were transfered to penassay broth and antigen type was examined by slide agglutination test. In order to determine the antigen of alternative phase, they were transfered farther to MGA stabs which contain antiserum to the expressed antigen and the antigen types of the swarms grown from them were examined by slide agglutination after transfering to penassay broth.

The results obtained were summarized in Table 1 and 2. From these table it is clear that in SW1043 b(1,2) --x SW1053 a(c) all of the transduction types

are (a)b; that means $H_1{}^b$ is transduced and replaced to $H_1{}^{c}$. In SW1053 a(c) ---x SW1043 b(1,2), $H_1{}^a$ is transduced and replaced to $H_1{}^b$. In both cases, simultaneous transduction of two H_1-loci was not found out.

DISCUSSION.

There are two feature on the result of present experiment:

(1). Only one of two antigen factors was transduced in every case.

(2). Only one type of transduction was observed in each experiment.

The possible explanations of the 1st phenomenon are classified as follows:

1). Phage incorporate at most only one H_1 factor from the donor.

a). Two H_1 of the donor locate on a same chromosome, but their distance is great and there is few or no chance th be incorporated in a same phage. (Two loci must be on the opposite side of Fla_{666} locus or Fla_{666} must be duplicate too.)

b). The donor is heterogenote on H_1-locus. (H_1-Fla_{666} must be heterogenote.)

2). Only one of the H_1-loci of the recipient can replacable at a time.

a). Two H_1-loci of the recipient are not allelic with two H_1-loci of the donor, (e.g. $\frac{c\ \ a}{b\ \ 1,2}$).

b). The recipient is heterogenote on H_1-locus.

The method to distinguish 1) from 2) is: (when SW1053 is donor)

b ---x SW1053 a:c ---> SW1053 a:b

i ---x SW1053 a:b ---> SW1053 i:b

SW1053 i:b ---x SW1053 a:c

---> if only i:c or a:b ----- 1)
---> if i:b appear ----- 2).

The methods to distinguish b) from a) are:

I. To test whether segregation of H_1 occurs or not.

(e. g. SW1053 a(c) --> Singlecolonies --> Transfer to anti-a MGA stab --> Look for stabs which donot grow swarms)

II. To test whether there are remarkable difference of transduction efficiency between two H_1-loci or not.

Now, we'll turn to the consideration of the 2nd phenomenon. Theoretically possible combinations produced from the present experiment are:

1) ab 2) a(1,2) 3) (c)b 4) (c)(1,2)

However, only 2) was found out on "a(c) --x b(1,2)" and 1) was found out on "b(1,2) --x a(c)". The disappearance of 1) in the former and of 2) in the later may well be explained by the selection of antiserum, because ab has b-phenotype and a(1,2) has a-phenotype as the results of experiment have shown.

There are several possibilities on the disappearance of 3) and 4) in both experiments:

1). Sample was not enough to detect all combination type; if experiment was repeated , type 3 and 4 will be found out.

2). Cytoplasmic condition determines the phase in b(c) and (c)(1,2).

3). Recombination does not occur at random between four loci. (e.g. b(c) does not appear as (1,2) is not replaced by (c), or a is not replaced by b.)

Present experiment is not enough to discuss in detail about these possibilities. For the further discussion and theoretical developement, more quantitative mutual transduction experiment using SW1053 (a)c and SW1043 (b)1,2 as well as SW1053 a(c) and SW1043 b(1,2) must be necessary.

SUPPLEMENTALLY DISCUSSION.

Each antigen factors of the transduction clon b(a), obtained from SW 1043 ---x SW1053, were H_1 originally. According to the hypothesis proposed from transductions between single phase cultures of H_1H_2-diphasic strains, both factors which control b or a must be stable and transduction type must become monophasic. The present experiment has shown b(a) produce swarm which has the phenotype of alternative phase oftenly on antiserum-MGA stab selection; that is, b(a) seems to be not monophasic but diphasic. This suggest the possibility that the mutability of H-loci is not owned by loci themselves but affected by some other factor (or factors ?). For the solution of this problem, the method to measure the rate of phase variation quantitatively must be established first. For this purpose, application of chemostat technique may be most useful.

Table 1.

Transduction from SW1043 b(1,2) to SW1053 a(c).

Donor	SW1043 b (FA 86)	———	SW1043 b (FA 86)	SW1043 b (FA 86)
Recipient	———	SW1053 a	SW1053 a	SW1053 a
Selective media	Anti-a,c MGA stab	"	"	plain MGA stab
Cell growth	-	+	+	+
No. of stabs grown / total swarms / no. of stabs	0 / 2	0 / 2	10 / 10	2 / 2
Antigens of the transductions	———	———	all of 10 are b(a)	a(c)

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Table 2.

Transduction from SW 1053 a(c) to SW1043 b(1,2).

Donor	SW1053 a	———	SW1053 a	SW1053 a
Recipient	———	SW1043 b	SW1043 b	SW1043 b
Selective media	Anti-b,1,2 MGA stab	"	"	plain MGA stab
Cell growth	-	+	+	+
No. stabs grown / total swarms / no. of stabs	0 / 4	7 / 12	9 / 10	4 / 4
Antigens of the transductions	———	all Of 7 are z^{33}(1,2)	3 are a(1,2) 6 are z^{33}(1,2)	b(1,2)

APPENDIX.

I. ON A POSSIBILITY OF THE PRESENCE OF 3RD H-LOCUS IN $SW1049G_3$

$SW1049G_3$ has H-antigens 1,2 and enx; they are both phase-2 antigens. In order to test whether phase-1 antigen is present in inhibited state as 3rd factor,or not, penassay broth culture of $SW1049G_3$ were selected by anti-1 2,-enx MGA stabs.

The experimental procedure is as follows: stab culture ---- streak on EMB-galactose plate ---- single colony isolation ---- grow in penassay broth ---- drop 0.05 ml to each anti-1,2,-enx MGA stab (prepared by addition of 0.05 ml of 1/100 diluted sera in each stab).

The experiment were repeated twice using 10 stabs in each experiment. Antigen types of the cultures in the first experiment were all 1,2,and in the second experiment,5 of them were 1,2 and the other 5 are enx. As far as tested, no growth of swarms were observed in them.